Traffic-Related Air Pollution and Parkinson's Disease in Denmark: A Case–Control Study

**Beate Ritz, Pei-Chen Lee, Johnni Hansen,
Christina Funch Lassen, Matthias Ketzel, Mette Sørensen,
and Ole Raaschou-Nielsen**

Traffic-Related Air Pollution and Parkinson's Disease in Denmark: A Case–Control Study

Beate Ritz,[1,2] Pei-Chen Lee,[3] Johnni Hansen,[4] Christina Funch Lassen,[4] Matthias Ketzel,[5] Mette Sørensen,[4] and Ole Raaschou-Nielsen[4]

[1]Department of Epidemiology, University of California at Los Angeles School of Public Health, Los Angeles, California, USA; [2]Department of Neurology, School of Medicine, University of California at Los Angeles, Los Angeles, California, USA; [3]Department of Health Care Management, College of Healthcare Administration and Management, National Taipei University of Nursing Health Sciences, Taipei, Taiwan; [4]Danish Cancer Society Research Center, Danish Cancer Society, Copenhagen, Denmark; [5]Department of Environmental Science, Aarhus University, Roskilde, Denmark

Address correspondence to Pei-Chen Lee, Dept. of Health Care Management, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan. 89, Nei-Chiang St. Wan-Hua Dist, Taipei, 10845, Taiwan. E-mail: pcl0807@gmail.com

Running title: Air pollution and Parkinson's disease

Abstract

Objective: Very little is currently known about air pollutants' adverse effects on neurodegenerative diseases even though recent studies have linked particulate exposures to brain pathologies associated with Parkinson's and Alzheimer's disease. Here we investigate long-term exposure to traffic-related air pollution and Parkinson's disease.

Methods: In a case-control study of 1,696 Parkinson's disease (PD) patients identified from Danish hospital registries and diagnosed 1996-2009 and 1,800 population controls matched by gender and year of birth we assessed long-term traffic-related air pollutant exposures (NO_2) from a dispersion model, using residential addresses from 1971 to the date of diagnosis or first cardinal symptom for cases and the corresponding index date for their matched controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated with logistic regression adjusting for matching factors and potential confounders.

Results: We found ambient air pollution from traffic sources to be associated with risk of PD, with a 9% higher risk (95% CI: 3, 16.0%) per interquartile range increase (2.97 μg/m^3) in modeled NO_2. For participants living for 20 years or more in the capital city odds ratios were larger (OR= 1.21; 95% CI: 1.11, 1.31) than in provincial towns (OR=1.10; 95% CI: 0.97, 1.26), while there was no association among rural residents.

Conclusions: Our findings raise concerns about potential effects of air pollution from traffic and other sources on the risk of PD, particularly in populations with high or increasing exposures.

Introduction

Air pollution is ubiquitous and particulate exposure levels are currently rising to

unprecedented levels in some emerging economies with traffic sources being major contributors.

Air pollutants' adverse effects on respiratory and cardiovascular health are well-documented

(Andersen et al. 2012; Anderson et al. 2012; Lim et al. 2012; Shah et al. 2013), yet very little is

currently known about the effects they may have on the aging brain. Parkinson's disease (PD) is

the second most common neurodegenerative disorder engendering great human costs in aging

populations (Huse et al. 2005), and recent evidence suggests that air pollution may act on

biologic pathways contributing to PD. Specifically pathologic studies of the human brain and

some animal experiments reported neuroinflammation, oxidative stress and dopamine system

related neurotoxicity with air pollution exposures (Block and Calderon-Garciduenas 2009;

Calderon-Garciduenas et al. 2010; Cannon et al. 2009; Levesque et al. 2011; Yamin et al. 2003).

Previously, researchers found neuropathological lesions in feral dogs living in Mexico

City, as well as inflammation of the olfactory bulb and deficits in olfaction in Mexican children

residing in highly polluted areas (Calderon-Garciduenas et al. 2008a; Calderon-Garciduenas et al.

2010). It has recently been proposed that the olfactory bulb may provide an anterograde entry

passage for pathogens and xenobiotics – including particles – along the olfactory tract i.e. a nose-

to-brain route that bypasses the protective blood brain barrier (Hawkes et al. 2009; Lucchini et

al. 2012). Interestingly, the Mexican researchers also detected alpha-synuclein neuronal

aggregates, characteristically found in Lewy-bodies of patients with PD, as well as increased

oxidative stress markers in brainstem nuclei of autopsied young people who died suddenly and

had been exposed to Mexico city's air pollution (Calderon-Garciduenas et al. 2008b). Misfolded

α-synuclein proteins furthermore were recently found to be transmissible within the brain, i.e. to

spread from affected to unaffected neurons by seeding misfolding of proteins (Desplats et al. 2009; Luk et al. 2012; Mougenot et al. 2012), a possible mechanism by which PD pathology (protein agglomeration) may reach the brain through the olfactory tract.

It is well-known that one of the early preclinical features of PD observed in almost all cases is the loss of the sense of smell (Doty 2012a, b; Levesque et al. 2011), and Braak's neuropathological staging of PD suggests that olfactory bulb neurons are among the first to display Lewy body pathology characteristic of PD (Braak et al. 2004)). Airborne ultrafine (nanosized) particles have been shown to translocate to mitochondria after endocytosis, to produce reactive oxygen species, and to pass through the blood brain barrier (Braak et al. 2004; Oberdorster and Utell 2002; Oberdorster et al. 2004). Major sources of population exposure to nanoparticles are internal combustion processes and traffic exhaust (Oberdorster and Utell 2002). While population monitoring of ultrafine particles is not available, land use regression (LUR) modeling relying on easier to measure gaseous indicators such as NOX and NO_2 have been shown to capture the fine spatial variation of pollutant mixtures from traffic well (Su et al. 2009).

Epidemiologic research of air pollution and dopaminergic neurodegeneration is just beginning. A cross–sectional study investigating manganese in air pollution suggested a small increase in PD prevalence and lower age at onset (Finkelstein and Jerrett 2007). An ecologic study of urban counties linked EPA recorded industrial emissions of copper and manganese to PD cases identified from Medicare records (Willis et al. 2010). Most recently researchers relied on the Harvard Nurses' Health cohort and assessed exposures first modelling levels of metals that are considered air toxics (Palacios et al. 2014a) and second fine and coarse particulate matter (Palacios et al. 2014b); these studies, however, did not find associations with PD. Here we are for the first time presenting data that associate PD with long-term (1971 onward) and fine scale

modeled exposure to NO_2 used as a marker of traffic-related air pollution mixtures in a

nationwide study in Denmark.

METHODS

Study Population

PD patients were identified from the Danish National Hospital Register (Lynge et al.

2011) between 1996 and mid-2009 and all were treated at 10 large neurological centers in

Denmark. This register has kept computerized records of all hospitalized patients in Denmark

since 1977 and also from outpatient clinics after 1994, including information on the patient

(name, the unique 10-digit personal number which is applied to all residents in Denmark), and

their primary as well as other diagnoses (Lynge et al. 2011). As described in more detail

(Wermuth et al. 2012), medically trained research staff supervised by a movement disorder

specialist reviewed medical records we obtained from the treating hospitals/outpatient clinics and

rigorously applied standard criteria to establish an idiopathic PD diagnosis (Hughes et al. 1992).

The diagnosis was based on all medical record information available at time of record retrieval,

including notes from private practitioners, and we required the presence of a minimum of two of

the following symptoms: resting tremor, bradykinesia, rigidity, and asymmetrical onset. We also

evaluated response to treatment with levodopa, signs of dementia and their timing, early falls,

severe symptomatic dysautonomia, and sudden symptom onset, supra-nuclear gaze palsy,

hallucinations unrelated to medication, and records for computed tomography scans, DaTSCANs,

or magnetic resonance scans. The occurrence of the first cardinal (motor) symptom noted on the

medical record or – if missing – the first known date of hospitalization/outpatient clinic visit due

to PD was the reference date for calculating time dependent exposures for cases and matched

controls. From a list of 2,762 initially eligible patients, we removed 179 subjects without

idiopathic PD (iPD) according to medical records we received prior to interview, 20 without

medical records to confirm diagnoses, and 497 (21.3%) who declined to participate. We further

excluded 238 interviewed cases after interview when the medical record review determined that

they did not suffer from iPD, leaving 1,828 confirmed iPD cases.

Initially, for each case we sampled 5-10 potential controls randomly from the Danish

Central Population Register, which covers historical information, including names, dates of birth,

death and immigration on all residents in Denmark . We aimed to enroll and interview one

control from each matched set. Thus, whenever a contacted control was willing to participate, no

other potential control from the same set was contacted. Controls were required to not have PD

based on Danish National Hospital Register records prior to the date their respective case

received a PD diagnosis and to be alive for interview. They were assigned the same index date as

their respective case. Out of 3,626 eligible controls initially contacted, 1,909 (52.6%) completed

an interview. All participants were interviewed between January 2008 and December 2010, to

obtain information on possible confounding variables including education, tobacco smoking

history and family history of PD.

Written informed consent was obtained from all subjects, and the study protocol was

approved by the UCLA Institutional Review Board and by the Danish Data Protection Agency

and the Ethical committee for the Copenhagen Region (H-D-2007-0009).

Address Geocoding and Air Pollution Exposure Assessment

We retrieved all addresses of study participants from 1 January 1971 onwards from the

Central Population Registry relying on the unique personal identification number for Danish

citizens including the dates of moving to and leaving from each address prior to index date.

Addresses, identified by municipality code, street code and house number, were linked to the

Danish Address Register to obtain geographical coordinates at the front door of the house. The

precision of the geographical coordinates was high, lying within 5 meters for the majority of

addresses and we successfully geocoded and estimated air pollution exposures at 88% of all

addresses. The geocodes in OSAK - the address/GIS registry used to translate address codes to

geocodes - refer to the middle of a building. If there are multiple addresses in one building, the

geocodes reflect the location of the front door of the specific address. The accuracy in OSAK is

reported as: 95-98% of the geocoding has accuracy better than 10 meters and for the majority of

these the accuracy is better than 5 meters. For 2-5% of the addresses, geocodes are calculated for

the center point of the land parcel registered (the piece of land on which the building is situated).

Thus the accuracy depends on the size of the parcel; for single-family houses the precision will

typically be better than 50 m.

We employed a GIS-based dispersion modeling system (AirGIS;

http://envs.au.dk/en/knowledge/air/models/airgis/) to estimate subjects' exposure to well

established surrogates of traffic-generated air pollution (NO_2 ,NO_x, CO) averaging over the

period starting in 1971 up to the participant's index date. Specifically, we calculated air

pollution estimates for each address as the sum of: (1) local air pollution from street traffic,

calculated from traffic intensity and type, emission factors for the car fleet, street and building

geometry and meteorology; (2) urban background, calculated from data on urban vehicle

emission density, city dimensions and building heights; and (3) regional background, estimated

from trends at rural monitoring stations and from national vehicle emissions. With the geocode

of an address and a specified year as the starting point, the AirGIS system automatically

generates street configuration data for the street pollution model, including street orientation,

street width, building heights in wind sectors, amount of traffic, speed and type as well as other required data.

We estimated historical traffic-related air pollutant exposures based on AirGIS estimates for hourly air pollution concentration for NO_2, NO_x , and CO at each address and calculated average concentrations between 1 January 1971 and the index date for each participant. We only included in analyses participants for whom the residential addresses were known and geocoded and allowed us to generate air pollution measures for 80% or more of the period between 1 January 1971 and the index date – 6% of cases and 5% of controls did not fulfill this criterion-, i.e. a total of 1,696 (93%) cases and 1,800 (94%) controls were included in our analyses. For those missing pollutant data for <20% of the period, we calculated exposure averages based only on days with non-missing data.

Statistical Methods

Exposure measures for the air pollutants were modeled as continuous (per interquartile range increase). We also investigated the shape of the exposure-response function between air pollution and PD using percentiles of exposure and splines with 2-knots (smoothers). Exposure lagging, i.e., excluding exposures that occurred within certain periods (5 or 10 years) prior to disease onset or interview, were explored to address concerns that exposures in years immediately preceding the PD diagnosis may not be etiologically relevant. Lag times of 5 and 10 years cover the latency period range most often suggested for pre-motor PD (Siderowf and Lang 2012). In order to include the largest possible number of cases and controls and to conduct stratified analyses, we broke the matches and used unconditional logistic regression models to obtain odds ratios and confidence intervals. We selected potential confounding variables using causal diagram methods as well as the more than 10% change in effect estimate criteria (Mickey

and Greenland, 1989). Thus, in our models we adjusted for the matching variables, birth year,

gender, and age at index date, and also for other potential confounding risk factors obtained in

interviews, such as education (basic, vocational, and higher education), smoking (smoking status

(never, ex-smoker, current smoker) and pack-years of smoking), family history of PD (first

degree relative with PD history diagnosed by a doctor, yes/no), and job history of air pollution

related exposures (i.e. any history of truck or bus drivers, traffic police, tractor, gas station

workers, and mechanic, asphalt workers). In adjusted models, missing covariate data was

excluded i.e., we conducted case analysis. Controls retained the index date they were originally

assigned even after matching was broken. Smoking status and all other exposures were defined

as life-time exposure before index date. In sensitivity analyses, we examined air pollution effects

by smoking status, gender, both cases and controls age at index date before or after age 60, and

location of the residence (Copenhagen and Suburbs, Provincial cities, rural towns and villages) at

different points in time (lag time), for those diagnosed most recently, i.e. between 2005 and

2009.. We assessed effect modification in stratified analyses and by entering product terms into

our logistic regression models. The interaction p-values were based on a log likelihood ratio test.

A one-sided p-value < 0.05 was considered statistically significant.

Results

The PD patients included in this study were on average 62 years of age at first symptom

onset or diagnosis, more were male than female (60-40%), half had received vocational training

and about a quarter a higher level of education; in all of these characteristics controls and cases

were quite similarly (Table 1). However, PD patients were more often never and former smokers

and also somewhat less likely to be born and living in Copenhagen than controls. Our exposure

estimates covered on average of 30.7 years of traffic related air pollution exposure. We focus on

results for NO_2 as a marker of traffic pollution mixtures, as it is most commonly reported in air

pollution research; NO_2 was highly correlated with NO_x and CO (Pearson correlation coefficients

of 0.92 and 0.81, respectively, and 0.85 for NO_x and CO).

Mean concentration of NO_2 in Copenhagen (mean=16.83 $\mu g/m^3$) is higher than

concentrations in provincial cities (mean=12.63 $\mu g/m^3$) and rural areas (mean=12.11 $\mu g/m^3$)

(Table 2). For each IQR (2.97 $\mu g/m^3$) increase in long-term exposure to NO_2, we estimated a 9%

increase in PD risk (OR=1.09; 95%CI: 1.03, 1.16) adjusting for year of birth, gender, age at first

cardinal symptom, education, smoking, family history of PD, and job history of indicators for

exposure to traffic pollution (Table 3). Using categories of exposure based on percentiles, we

estimated an adjusted OR of 1.08 (95%CI: 0.90, 1.30) for exposures in the 25-75th percentile and

an OR of 1.22 (95%CI: 0.99, 1.51) above the 75th percentile for NO_2 as compared to the <25th

percentile suggesting that our results were not unduly influenced by the highest exposures; yet,

NO_2 exposures above the 95th percentile suggested a strong association (OR=1.92 ; 95%CI: 1.32,

2.80) based on 103 cases and 72 controls in this category. Spline models with 2-knots

(smoothers) showed an overall increase in risk with increasing exposure above the mean NO_2

concentration, however, with some scatter in the lower end of the exposure range, and imprecise

estimates in the upper part of the range due to sparse data (Figure 1). Odds ratios were

comparable between men and women (Table 3). Associations were slightly stronger in current

smokers than in never and ex-smokers, but the interaction with smoking status was not

significant overall (p-interaction = 0.31). Point estimates appeared somewhat larger for patients

experiencing first symptoms or being diagnosed prior to age 60 compared with those diagnosed

at older ages but confidence intervals largely overlapped (interaction p=0.40). Restricting our

analyses only to participants with diagnosis or index dates from 2005 to early 2009 (N=1,351) had little effect on the OR, though the estimate was less precise (adj. OR = 1.08; 95% CI: 0.95, 1.24). None of the differences in estimates presented in Table 3 for different subgroups were formally statistically significant when testing for interactions in our regression models.

Associations were similar in size for those born in Copenhagen and its suburbs and provincial towns, but the association with the same increase in NO2 was null for those born in rural areas (Table 3). The pattern was similar when stratifying by place of residence at the diagnosis or index date (Table 3) or 20 years before the diagnosis or index date (results not shown). Results did not change when we lagged NO_2 by 5 and 10 years prior to the diagnosis/index date (Table 3). Finally, associations were similar for IQR increases in CO (fully adjusted OR = 1.13; 95% CI 1.06, 1.21 for a 0.12 ppm increase) and NO_x (adj. OR = 1.06; 95% CI: 1.02, 1.11 for a 7.10 ppm increase).

Discussion

To our knowledge, this is the first study to suggest that ambient air pollution due to traffic related sources increases the risk of Parkinson' disease. This association was observed among those having been born or living in the capital city or provincial towns, while rural residents seemed to not be at higher risk according to our modelled air pollution measures. Also associations were positive whether participants lived in these places at birth, 20 years prior to or at time of diagnosis. Estimated effect sizes were largest for those living in the Capital Copenhagen.

Air pollution including that from traffic related sources is well-known to adversely affect human health and has recently been shown to contribute to processes that may damage the brain (Block and Calderon-Garciduenas 2009; Calderon-Garciduenas et al. 2008b; Campbell et al.

2005; Hartz et al. 2008; Mills et al. 2009; Schwartz et al. 2005; Sun et al. 2005). In animal

experiments ultrafine particulates from combustion (<0.1 μm in aerodynamic diameter), the main

contributor of traffic-related air pollution, were shown to reach the brain, cause inflammation,

and act as neurotoxins (Elder et al. 2006; Oberdorster et al. 2004; van Berlo et al. 2010). In

terms of human experimental studies, studies using autopsied human brain samples found

significantly higher cyclooxygenase-2 expression, an inflammatory mediator, in the frontal

cortex and hippocampus and greater neuronal and astrocytic accumulation of α-synuclein in

neurons, glial cells and/or blood vessels in subjects with lifelong exposure to high levels of air

pollution compared to controls from a relatively clean environment (Calderon-Garciduenas et al.

2004; Calderon-Garciduenas et al. 2010). These findings are important because the α-synuclein

protein and its aggregates are major components of Lewy-bodies, a PD hallmark feature. We

reported recently that associations between pesticides and PD were stronger among study

participants who carried genetic variants linked to higher expression of the α-synuclein gene

(Gatto et al. 2010).

Two out of four previous human studies investigating air pollution effects on PD risk

focused solely on metals classified as air toxics. A Canadian study (Finkelstein and Jerrett 2007)

did not find associations with a land use regression derived traffic related pollution measure for

NO_2 but reported small positive associations with PD for manganese, a metal that has been

related to a syndrome with Parkinsonian features called 'manganism' distinct from idiopathic PD

(Rivera-Mancia et al. 2011). The authors relied on administrative records for PD drug use and

diagnosis codes but did not validate PD diagnoses and included prevalent cases. This study also

assess exposures only for the brief period from 1992–1999 during which cases were identified

from the database i.e. around the time of diagnosis or even after for prevalent cases. The

Medicare beneficiary study in the US (Willis et al. 2010) used a coarse county scale exposures model and identified manganese and copper from industrial releases as potential risk factors for PD. Recent publications from the Nurses' Health Study did not find evidence that any of the investigated metals known as air toxics (Palacios et al. 2014a) or the fine and coarse particulate matter exposures they modelled (Palacios et al. 2014b) increased PD risk. However, the exposure period for which modeled residential air pollutant exposures were available overlapped almost entirely with what is now believed a lengthy (10-20 years) preclinical period of PD (Siderowf and Lang 2012) i.e. did not pre-date this preclinical period. Thus, our exposure model for Denmark covered a much longer and possibly more relevant period of participants' lifetime. Furthermore, the EPA hazardous air pollution (HAPs) model the Nurses' Health Study employed is spatially coarse, i.e. too coarse to assess mobile sources locally, and while it captures industry reported releases of air toxics it does not provide the same data as a finer scale air toxics monitoring network (U.S. EPA 2011). . The PD risk estimate for a continuous measure of $PM_{2.5}$ in the Nurses' Health Study was 1.08 (95%CI: 0.81,1.45) per 10 $\mu g/m^3$, an imprecise estimate that suggests a small positive association.

While Danish air pollution levels are lower than those reported for Southern and Eastern Europe, they are similar to Western industrial nations in terms of the contributions of vehicular traffic exhaust which continues to be a major source of air pollution in Denmark (Eeftens et al 2012). Historical and long-term traffic-related air pollution in Denmark modeled as in our PD study have previously been associated with increased risk of developing lung cancer among non-smokers and also with brain tumors (ICD-7 193) in the Danish Diet Cancer and Health cohort (54,304 participants) that relied on the Danish Cancer Registry to identify cancers between enrolment in 1993-1997 until 2006, and traced their residential addresses from 1971

onwards in the Central Population Register   (Raaschou-Nielsen et al. 2011a; Raaschou-Nielsen

et al. 2011b).

We were unable to directly measure traffic related air pollution, but the AirGIS

dispersion models we employed provided us with estimates of NO_2 as indicators for traffic

pollution mixtures. The AirGIS model for assessing traffic-related air pollution exposures in

Denmark uses various detailed traffic parameters with high temporal and address-level spatial

resolution validated in several studies (Berkowicz et al. 2008; Ketzel et al. 2011; Raaschou-

Nielsen et al. 2000). For example, the correlation between modeled and measured half-year mean

NO_2 concentrations at 204 locations in the greater Copenhagen area had a coefficient of 0.90

(Berkowicz et al. 2008); and the correlation between modeled and measured 1-month mean

concentrations of NO_x and NO_2 over 12 years (1995–2006) on a busy street in Copenhagen

(Jagtvej, 25 000 vehicles per day, street canyon) had coefficients of 0.88 and 0.67 respectively.

Furthermore, residential addresses from the Danish Address Register enabled us to estimate on

average over 30 years of air pollution exposures while accounting for mobility further reduceing

exposure misclassification. Our study participants resided throughout Denmark and this

residential diversity provided high variability in exposure from air pollution due to traffic

sources. However, we lacked workplace address information and were unable to take into

account indoor air quality.

A major strength of the study is our ability to select PD patients and controls from most

of Denmark relying on the National Hospital Register that identifies all Danish hospitalized and

outpatient clinic patients. Further, health care including hospitals are free for all citizens in

Denmark, thus avoiding biases from differential access to health care. Also, our controls were

identified in a population-based manner from the Central Population Register. We were able to

14

minimize disease misclassification due to thorough abstraction of medical records for all

hospitalized PD patients (15% of all records of eligible patients revealed a diagnosis others than

idiopathic PD) (Wermuth et al. 2012). The participation rate in interviews was high among the

patients (80.8%), but more moderate in controls (52.6%). Generally, selection bias could occur if

a higher proportion of non-responding controls than cases were exposed to high air pollution.

Through record linkage we learned that twice as many nonparticipants than participants had died

as of March 2013 (13.0% vs. 5.9% controls and 38% vs. 18% cases). In addition, slightly more

non-participants than participants were hospitalized with diseases that may be affected by air

pollution specifically ischemic heart disease (14.8% *vs*. 13.4% controls and 21.3% vs. 16.1%

cases). Thus, we cannot rule out selection (or survivor) bias in our study due to the influence of

air pollution related health status on participation.. Male gender, older age, and smoking are

strong risk factors for cardiovascular and pulmonary diseases. Thus, if our air pollution

associations were due to selection bias removing more controls highly exposed to air pollution

with cardiovascular and pulmonary risk factors, we would have expected air pollution to be more

strongly associated with PD in sensitivity analyses for such higher risk groups; i.e. smokers,

older, and male subjects. However, we found little evidence for this in our subgroup analyses i.e.

we found no differences for traffic-related air pollution estimates for men compared with women,

slightly stronger effects in current than in former and never-smokers, but weaker effects in those

diagnosed with PD at an older compared with a younger age (>60 years). We also did not see

any changes in estimated effects when adjusting for occupations related to air pollution

exposures such as drivers (data not shown). Effect estimates for residing in Copenhagen and its

suburbs were stronger than for other cities and we did not identify risk increases with traffic

exposures among rural residents. This difference may be related to the low levels and/or low

geographical variation in traffic-related air pollution in rural Denmark.

PD patients living in Copenhagen were slightly under-sampled by the National Hospital Register;

i.e. 30.7% of controls but only 25.7% of cases were living in the capital and its suburbs at time of

diagnosis. One explanation might be that more patients who live in Copenhagen are treated

exclusively by private practitioners not reporting to the hospital registers that capture an

estimated 80% of all Danish PD cases i.e. the patients who are treated at clinics and hospitals

(personal communication L. Wermuth). If we missed cases who lived in less polluted areas this

could have biased our results away from the null.

In summary, the large sample size of our study together with our exposure assessment

techniques covering a very long time span allowed us to conduct an investigation that suggests

long-term impacts of air pollution on PD risk. Exposure to traffic related air pollution affects

large populations in most countries and may be rising further in the near future due to worldwide

urbanization trends. This human data is supported by results from animal experiments and

pathology studies and raises concern given the increase in vulnerable aging populations.

References

Andersen ZJ, Bonnelykke K, Hvidberg M, Jensen SS, Ketzel M, Loft S, et al. 2012. Long-term exposure to air pollution and asthma hospitalisations in older adults: A cohort study. Thorax 67:6-11.

Anderson JO, Thundiyil JG, Stolbach A. 2012. Clearing the air: A review of the effects of particulate matter air pollution on human health. J Med Toxicol 8:166-175.

Berkowicz R, Ketzel M, Jensen SS, Hvidberg M, Raaschou-Nielsen O. 2008. Evaluation and application of ospm for traffic pollution assessment for a large number of street locations. Environmental Modelling & Software 23:296-303.

Block ML, Calderon-Garciduenas L. 2009. Air pollution: Mechanisms of neuroinflammation and CNS disease. Trends Neurosci 32:506-516.

Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. 2004. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318:121-134.

Calderon-Garciduenas L, Reed W, Maronpot RR, Henriquez-Roldan C, Delgado-Chavez R, Calderon-Garciduenas A, et al. 2004. Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. Toxicol Pathol 32:650-658.

Calderon-Garciduenas L, Mora-Tiscareno A, Ontiveros E, Gomez-Garza G, Barragan-Mejia G, Broadway J, et al. 2008a. Air pollution, cognitive deficits and brain abnormalities: A pilot study with children and dogs. Brain Cogn 68:117-127.

Calderon-Garciduenas L, Solt AC, Henriquez-Roldan C, Torres-Jardon R, Nuse B, Herritt L, et al. 2008b. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol 36:289-310.

Calderon-Garciduenas L, Franco-Lira M, Henriquez-Roldan C, Osnaya N, Gonzalez-Maciel A, Reynoso-Robles R, et al. 2010. Urban air pollution: Influences on olfactory function and pathology in exposed children and young adults. Exp Toxicol Pathol 62:91-102.

Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C, et al. 2005. Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. Neurotoxicology 26:133-140.

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. 2009. A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 34:279-290.

Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. 2009. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein (vol 106, pg 13010, 2009). Proceedings of the National Academy of Sciences of the United States of America 106:17606-17606.

Doty RL. 2012a. Olfactory dysfunction in parkinson disease. Nat Rev Neurol 8:329-339.

Doty RL. 2012b. Olfaction in parkinson's disease and related disorders. Neurobiol Dis 46:527-552.

Eeftens M, Beelen R, de Hoogh K, Bellander T, Cesaroni G, Cirach M, et al. 2012. Development of Land Use Regression models for PM(2.5), PM(2.5) absorbance, PM(10) and PM(coarse) in 20 European study areas; results of the ESCAPE project. Environ Sci Technol 46: 11195-205.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, et al. 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114:1172-1178.

Fine PM, Sioutas C, Solomon PA. 2008. Secondary particulate matter in the United States: Insights from the particulate matter supersites program and related studies. J Air Waste Manag Assoc 58:234-253.

Finkelstein MM, Jerrett M. 2007. A study of the relationships between Parkinson's disease and markers of traffic-derived and environmental manganese air pollution in two Canadian cities. Environ Res 104:420-432.

Gatto NM, Rhodes SL, Manthripragada AD, Bronstein J, Cockburn M, Farrer M, et al. 2010. Alpha-synuclein gene may interact with environmental factors in increasing risk of Parkinson's disease. Neuroepidemiology 35:191-195.

Hartz AM, Bauer B, Block ML, Hong JS, Miller DS. 2008. Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and p-glycoprotein up-regulation at the blood-brain barrier. FASEB J 22:2723-2733.

Hawkes CH, Del Tredici K, Braak H. 2009. Parkinson's disease: The dual hit theory revisited. Ann NY Acad Sci 1170:615-622.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. 1992. Accuracy of clinical diagnosis of idiopathic parkinson's disease: A clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55:181-184.

Huse DM, Schulman K, Orsini L, Castelli-Haley J, Kennedy S, Lenhart G. 2005. Burden of illness in Parkinson's disease. Mov Disord 20:1449-1454.

Ketzel M, Berkowicz R, Hvidberg M, Jensen SS, Raaschou-Nielsen O. 2011. Evaluation of AIRGIS: A GIS-based air pollution and human exposure modelling system. Int J Environ Pollut 47:226-238.

Levesque S, Taetzsch T, Lull ME, Kodavanti U, Stadler K, Wagner A, et al. 2011. Diesel exhaust activates and primes microglia: Air pollution, neuroinflammation, and regulation of dopaminergic neurotoxicity. Environ Health Perspect 119:1149-1155.

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. Lancet 380:2224-2260.

Lucchini RG, Dorman DC, Elder A, Veronesi B. 2012. Neurological impacts from inhalation of pollutants and the nose-brain connection. Neurotoxicology 33:838-841.

Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. 2012. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338:949-953.

Lynge E, Sandegaard JL, Rebolj M. 2011. The Danish National Patient Register. Scand J Public Health 39(7 Suppl):30-33.

Mickey RM, Greenland S. 1989. The impact of confounder selection criteria on effect estimation. Am .J. Epidemiol. 129:125-137.

Mills NL, Donaldson K, Hadoke PW, Boon NA, MacNee W, Cassee FR, et al. 2009. Adverse cardiovascular effects of air pollution. Nat Clin Pract Cardiovasc Med 6:36-44.

Mougenot AL, Nicot S, Bencsik A, Morignat E, Verchere J, Lakhdar L, et al. 2012. Prion-like acceleration of a synucleinopathy in a transgenic mouse model. Neurobiol Aging 33:2225-2228.

Oberdörster G, Utell MJ. 2002. Ultrafine particles in the urban air: To the respiratory tract--and beyond? Environ Health Perspect 110:A440-441.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. 2004. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16:437-445.

Palacios N, Fitzgerald K, Roberts AL, Hart JE, Weisskopf MG, Schwarzschild MA, et al. 2014a. A prospective analysis of airborne metal exposures and risk of Parkinson disease in the Nurses' Health Study cohort. Environ Health Perspect 122:933-938.

Palacios N, Fitzgerald KC, Hart JE, Weisskopf MG, Schwarzschild MA, Ascherio A, et al. 2014b. Particulate matter and risk of Parkinson disease in a large prospective study of women. Environ Health 13:80.

Raaschou-Nielsen O, Hertel O, Vignati E, Berkowicz R, Jensen SS, Larsen VB, et al. 2000. An air pollution model for use in epidemiological studies: Evaluation with measured levels of nitrogen dioxide and benzene. J Expo Anal Environ Epidemiol 10:4-14.

Raaschou-Nielsen O, Andersen ZJ, Hvidberg M, Jensen SS, Ketzel M, Sorensen M, et al. 2011a. Air pollution from traffic and cancer incidence: A Danish cohort study. Environ Health 10:67.

Raaschou-Nielsen O, Andersen ZJ, Hvidberg M, Jensen SS, Ketzel M, Sorensen M, et al. 2011b. Lung cancer incidence and long-term exposure to air pollution from traffic. Environ Health Perspect 119:860-865.

Rivera-Mancia S, Rios C, Montes S. 2011. Manganese accumulation in the CNS and associated pathologies. Biometals 24:811-825.

Schwartz J, Litonjua A, Suh H, Verrier M, Zanobetti A, Syring M, et al. 2005. Traffic related pollution and heart rate variability in a panel of elderly subjects. Thorax 60:455-461.

Shah AS, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, et al. 2013. Global association of air pollution and heart failure: A systematic review and meta-analysis. Lancet 382:1039-1048.

Siderowf A, Lang AE. 2012. Premotor Parkinson's disease: Concepts and definitions. Mov Disord 27:608-616.

Su JG, Jerrett M, Beckerman B, Wilhelm M, Ghosh JK, Ritz B. 2009. Predicting traffic-related air pollution in Los Angeles using a distance decay regression selection strategy. Environ Res 109:657-670.

Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, et al. 2005. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.

U.S. EPA (U.S. Environmental Protection Agency). 2011. Technology Transfer Network Air Toxics: National Air Toxics Assessments. Available: http://www.epa.gov/nata/ [accessed 14 June 2015]

van Berlo D, Albrecht C, Knaapen AM, Cassee FR, Gerlofs-Nijland ME, Kooter IM, et al. 2010. Comparative evaluation of the effects of short-term inhalation exposure to diesel engine exhaust on rat lung and brain. Arch Toxicol 84:553-562.

Wermuth L, Lassen CF, Himmerslev L, Olsen J, Ritz B. 2012. Validation of hospital register-based diagnosis of Parkinson's disease. Dan Med J 59:A4391.

Willis AW, Evanoff BA, Lian M, Galarza A, Wegrzyn A, Schootman M, et al. 2010. Metal emissions and urban incident Parkinson disease: A community health study of Medicare beneficiaries by using geographic information systems. Am J Epidemiol 172:1357-1363.

Yamin G, Glaser CB, Uversky VN, Fink AL. 2003. Certain metals trigger fibrillation of methionine-oxidized alpha-synuclein. J Biol Chem 278:27630-27635.

Table 1. Demographic characteristics of the study population by PD status

Characteristic	**Cases** (N=1696)	**Controls** (N=1800)
Age (years)[a], mean ±SD	61.65±9.50	61.57±9.65
Gender, n (%)		
Female	688 (40.6)	733 (40.7)
Male	1008 (59.4)	1067 (59.3)
Education[b]**,** n (%)		
Basic education and high school (7-12 years)	402 (23.7)	410 (22.9)
Vocational training (10-12 years)	822 (48.6)	889 (49.6)
Higher education (≥13 years)	469 (27.7)	494 (27.5)
Smoking history[b]**,** n (%)		
Never smoked	841 (49.7)	642 (35.9)
Ex-smoker	711 (42.1)	788 (44.0)
Current smoker	139 (8.2)	360 (20.1)
Place of birth [b], n (%)		
Copenhagen and Suburbs	349 (21.2)	473 (27.0)
Provincial cities	926 (56.4)	903 (51.7)
Rural	367 (22.4)	373 (21.3)
Place of living 20 years before diagnosis[b], n (%)		
Copenhagen and Suburbs	435 (25.7)	544 (30.3)
Provincial cities	959 (56.6)	953 (53.1)
Rural	300 (17.7)	299 (16.6)
Place of living at diagnosis [b], n (%)		
Copenhagen and Suburbs	435 (25.7)	544 (30.3)
Provincial cities	959 (56.6)	953 (53.1)
Rural	300 (17.7)	299 (16.6)
Family history of PD, n (%)		
None	1460 (86.1)	1707 (94.8)
At least one first-degree relative diagnosed with PD	236 (13.9)	93 (5.2)
NO_2 (µg/m^3, 1971 to censoring) , mean ±SD	13.83±4.22	13.59±3.57

[a] Age at first cardinal symptom of PD patients and their matched controls
[b] missing: education (n=10), smoking (n=15), place of living 20 years before diagnosis (n=6), place of birth (n=105), and place of living at diagnosis (n=81)

Table 2. Descriptive statistics of average residential air pollution exposures

Pollutants	Mean ± SD	IQR	Min	Percentile 25th	50th	75th	95th	Max
Place of residence at index date overall								
NO$_2$ (µg/m^3)	13.71±3.90	2.97	9.80	11.35	12.24	14.32	21.44	43.26
NO$_x$ (µg/m^3)	21.00±13.02	7.10	13.46	14.73	16.49	21.83	42.12	181.55
CO (mg/m^3)	0.55±0.16	0.12	0.36	0.46	0.50	0.58	0.82	2.34
Place of residence at index date by place								
Copenhagen and Suburbs								
NO$_2$ (µg/m^3)	16.83±5.23	6.31	10.65	13.02	15.35	19.33	27.11	43.26
Provincial cities								
NO$_2$ (µg/m^3)	12.63±2.46	1.99	9.80	11.27	11.83	13.26	16.72	38.62
Country								
NO$_2$ (µg/m^3)	12.11±1.79	1.40	10.64	11.11	11.51	12.51	15.24	29.54

Table 3. Associations (ORs and 95% CIs) between log-term traffic-related air pollution exposure[a] and Parkinson's Disease

Model	N (case/con.)	Crude[b] OR(95%CI)	Adjusted model[c] OR(95%CI)	*P* for interaction [d]
All	1696/1800	1.05 (1.00, 1.11)	1.09 (1.03, 1.16)	
<25th percentile	418/440	1.00	1.00	*0.06e*
25-<75th percentile	849/915	0.98 (0.83, 1.16)	1.08 (0.90, 1.30)	
≥75th percentile	429/445	1.02 (0.85, 1.24)	1.22 (0.99, 1.51)	
Diagnosed from 2005 to early 2009	665/686	0.98 (0.90, 1.07)	1.08 (0.95, 1.24)	
Lagging exposures 5 years	1695/1800	1.02 (0.98, 1.06)	1.07 (1.02, 1.12)	
Lagging exposures 10 years	1691/1796	1.02 (0.98, 1.07)	1.08 (1.03, 1.13)	
Sex				*0.83*
Male	1008/1067	1.06 (0.99, 1.13)	1.09 (1.01, 1.18)	
Female	688/733	1.04 (0.96, 1.13)	1.09 (1.00, 1.19)	
Smoking status				*0.31*
Never smoked	841/642	1.07 (0.98, 1.16)	1.07 (0.98, 1.17)	
Ex-smoker	711/788	1.06 (0.98,1.15)	1.09 (1.00, 1.18)	
Current smoker	139/360	1.09 (0.96, 1.23)	1.23 (1.03, 1.46)	
Age at first cardinal symptom				*0.40*
<60	691/735	1.09 (1.01, 1.18)	1.16 (1.06, 1.27)	
≥60	1005/1065	1.02 (0.95, 1.09)	1.06 (0.98, 1.14)	
Place of birth				*0.41*
Copenhagen and Suburbs	349/473	1.11 (1.02, 1.21)	1.16 (1.05, 1.27)	
Provincial cities	926/903	1.10 (1.01, 1.20)	1.16 (1.05, 1.29)	
Rural	367/373	1.03 (0.89, 1.20)	0.99 (0.83,1.19)	
Place of residence at index date (diagnosis of case)				*0.14*
Copenhagen and Suburbs	435/525	1.16 (1.08, 1.25)	1.21 (1.11, 1.31)	
Provincial cities	959/908	1.09 (0.98, 1.22)	1.10 (0.97, 1.26)	
Rural	300/288	0.93 (0.71, 1.22)	0.93 (0.68, 1.27)	

[a] per 2.97 µg/m^3 increase
[b] un-conditional logistic model adjusted for age at diagnosis or index date, gender, and year of birth
[c] un-conditional logistic model adjusted for age at diagnosis or index date , gender, education, smoking status (never, ex-smoker, current smoker), pack-years of smoking, family history of PD, and year of birth, and job history
[d] interaction p-values were based on a log likelihood ratio test
[e] *p* for trend

Figure Legend

Figure 1. ORs and 95% CIs based on a spline model (with 2-knots) of NO_2, with each unit on the x-axis corresponding to a multiple of the IQR (2.97 $\mu g/m^3$) over the estimated range of exposures (9.80–43.26 $\mu g/m^3$, corresponding to 3.3 and 14.6 IQR units, respectively), relative to the mean NO_2 concentration (13.71 $\mu g/m^3$, corresponding to 4.6 IQR units). Model adjusted for age at diagnosis or index date, gender, education, smoking status (never, ex-smoker, current smoker), pack-years of smoking, family history of PD, and year of birth, and job history.

Figure 1.